Detection of Schistosoma DNA in genital specimens and urine

A comparison between five female African study populations originating from S. haematobium and/or S. mansoni endemic areas

Pillay, P; Downs, J A; Changalucha, J M; Brienen, E A T; Ramarokoto, C E; Leutscher, P D C; Vennervald, B J; Taylor, M; Kjetland, E F; Van Lieshout, L

Published in:
Acta tropica

DOI (link to publication from Publisher):
10.1016/j.actatropica.2020.105363

Publication date:
2020

Document Version
Publisher's PDF, also known as Version of record

Acta Tropica 204 (2020) 105363

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Detection of *Schistosoma* DNA in genital specimens and urine: A comparison between five female African study populations originating from *S. haematobium* and/or *S. mansoni* endemic areas

P. Pillay[a,i,1,*], J.A. Downs[b], J.M. Changalucha[c], E.A.T. Brienen[d], C.E. Ramarokoto[e], P.D.C. Leutscher[f,g], B.J. Vennervald[h], M. Taylor[i], E.F. Kjetland[i,j], L. Van Lieshout[d,1]

[a] Department of Biomedical and Clinical Technology, Durban University of Technology, South Africa
[b] Center for Global Health, Department of Medicine, Weill Cornell Medicine, New York, USA
[c] Department of Medicine, Bugando Medical Centre, Mwanza, Tanzania
[d] Department of Parasitology, Leiden University Medical Center, The Netherlands
[e] Department of Epidemiology, Institut Pasteur de Madagascar, Antananarivo, Madagascar
[f] Centre for Clinical Research, North Denmark Regional Hospital, Denmark
[g] Department of Clinical Medicine, Aalborg University, Denmark
[h] Section for Parasitology and Aquatic Pathobiology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark
[i] Discipline of Public Health Medicine, Nelson R Mandela School of Medicine, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa
[j] Norwegian Centre for Imported and Tropical Diseases, Department of Infectious Diseases Ullevaal, Oslo University Hospital, Oslo Norway

ARTICLE INFO

Keywords:
Schistosoma
Female Genital Schistosomiasis (FGS)
Laboratory diagnosis
real-time PCR
Cervico-vaginal lavage, microscopy

ABSTRACT

Female Genital Schistosomiasis (FGS) is a neglected disease affecting millions, however challenging to diagnose. This explorative descriptive study compares *Schistosoma* real-time PCR analysis of cervico-vaginal lavages (CVL) with corresponding urine and stool samples of 933 women from five different previously described study populations. Sampling included 310 women from an *S. mansoni* endemic region in Mwanza, Tanzania and 112 women from a nearby *S. haematobium* endemic region. Findings were compared with samples collected from *S. haematobium* endemic regions in South Africa from 394 women and from 117 women from Madagascar of which 79 were urine pre-selected microscopy positive cases from highly-endemic communities and 38 were urine microscopy negatives from a low-endemic community. As anticipated, urine and stool microscopy and gynecological investigations varied substantially between study populations; however, the same *Schistosoma* real-time PCR was performed in one reference laboratory. *Schistosoma* DNA was detected in 13% (120/933) of the CVL, ranging from 3% in the *S. mansoni* Tanzanian endemic region to 61% in the pre-selected Malagasy urine microscopy positive cases. Detectable *Schistosoma* DNA in CVL was associated with *Schistosoma* DNA in urine but not with microscopic detection of eggs in urine or by cytological examination. This study confirmed real-time PCR for the detection of *Schistosoma* DNA in gynecological samples to be a valuable diagnostic tool to study the distribution of FGS within schistosomiasis endemic areas.

1. Introduction

Schistosomiasis is a neglected tropical disease of poverty, in 2017 it was estimated that approximately 220 million people worldwide had schistosomiasis (WHO, 2019). Women and girls in sub-Saharan low-income countries are especially at risk for female genital schistosomiasis (FGS). Beginning early in life, eggs in the genital tract of women can cause pathognomonic findings that include contact bleeding, homogenous sandy patches, grainy sandy patches and rubbery papules, manifestations also known FGS (Kjetland et al., 2012). While *S. haematobium* has been described as more frequently associated with FGS, cases of *S. mansoni* associated FGS have also been reported from both Brazil and northern Tanzania (Downs et al., 2011; Gonçalves Amorim et al., 2014; Poggensee et al., 2001a). Studies have indicated a higher

Abbreviations: FGS, female genital schistosomiasis; PCR, polymerase chain reaction; CVL, cervico-vaginal lavage; DNA, Deoxyribose nucleic acid; HIV, Human immunodeficiency virus

* Corresponding author at: Department of Biomedical and Clinical Technology, Durban University of Technology, South Africa.
E-mail address: pillayp@dut.ac.za (P. Pillay).

[1] Both authors contributed equally to the manuscript

https://doi.org/10.1016/j.actatropica.2020.105363
Received 29 March 2019; Received in revised form 27 January 2020; Accepted 28 January 2020
Available online 05 February 2020

susceptibility to HIV infection in women with FGS (Downs et al., 2017; Kjetland et al., 2006; Wall et al., 2018). It has been suggested that intravaginal lesions present in *S. haematobium* infected girls before sexual debut may make them more susceptible to HIV than their uninfected peers (Hegertun et al., 2013). Although *S. mansoni* ova can be found in the genital tract, the extent that *S. mansoni* infection also increases the risk of HIV acquisition and whether this is due to the same mechanisms as for *S. haematobium* are not completely clear (Downs et al., 2017; Ssetaala et al., 2015). The potential association between *S. mansoni* infection and FGS needs to be explored further.

Diagnosing schistosomiasis and *Schistosoma*-related morbidity can be difficult, especially when the ova lodge in different sites within the human body and the associated lesions are not always detectable (Kjetland et al., 2012). The presence or absence of *Schistosoma* eggs in urine or stool does not always reliably reflect the presence or absence of eggs within internal organs. Concerning involvement of the female genital tract, the reference standard diagnostic method is the microscopic detection of *Schistosoma* eggs in a perfectly acquired biopsy (Poggensee et al., 2001b). However sampling the cervix is not an appropriate approach for women and girls or for those at risk of being exposed to HIV (Kjetland et al., 2005; Norseth et al., 2014). Alternatively, PCR-based detection of *Schistosoma* DNA in cervico-vaginal lavage (CVL), being a non-invasive genital specimen, has been explored in several studies as a diagnostic indicator for FGS (Galappaththi-Arachchige et al., 2018; Kjetland et al., 2012; Pillay et al., 2016; Randrianasolo et al., 2015). So far, the results have been promising and suggest that laboratory detection of *Schistosoma* DNA in CVL has diagnostic potential as it seems to reflect genital involvement of the *Schistosoma* infection. At the same time, not all diagnostic aspects of the detection of *Schistosoma* DNA in gynecological specimens have been fully explored as previous studies focused mainly on the laboratory analysis of CVL and urine samples of *S. haematobium* infected cases.

In this study, the performance of a standardized real-time PCR procedure for the detection of *Schistosoma* DNA was compared amongst women from five different study populations in three different countries in Africa, namely Tanzania, South Africa and Madagascar (Downs et al., 2011; Pillay et al., 2016; Randrianasolo et al., 2015). Besides microscopy of urine, stool and Pap-smears, real-time PCR was performed on a urine, stool and CVL sample from each participating woman. This extensive laboratory sample analysis opens the possibility to further establish the diagnostic potentials of *Schistosoma* DNA detection in CVL, including CVL from a predominantly *S. mansoni* endemic population.

2. Materials and methods

2.1. Study population, ethics, recruitment and sampling

In total CVL samples from 933 women were included in the study. Fig. 1 presents the flowchart demonstrating the five study regions, where the samples were collected. Participants with incomplete data or missing samples were excluded, hence minor differences with the original publications. The geographical location of the three countries included in the study is depicted in Fig. 2. An informed consent was part of each enrollment procedure. At each site, only sexually active, consenting women were invited into the study. Women who were severely ill, virgins or pregnant were excluded. Ethical approval was obtained for each project and has been described in full detail in the original study descriptions (Downs et al., 2011; Pillay et al., 2016; Randrianasolo et al., 2015). Additional details about study design, study populations and procedures used, are briefly summarized below (Downs et al., 2011; Holmen et al., 2015; Randrianasolo et al., 2015).

Tanzania. The study was nested within a larger survey conducted during 2009–2010 at eight rural primary clinics in Mwanza, south of Lake Victoria. This region was reported to have a *Schistosoma* prevalence of between 50 and 90% among schoolchildren (Clements et al., 2006; Downs et al., 2011). Six of the clinics were located near to the Lake in a region predominantly endemic for *S. mansoni* (region 1A), while the two other clinics were located more to the South, where only *S. haematobium* infections is endemic (region 1B) (Downs et al., 2011). A total of 457 eligible women attending a free cervical cancer-screening program were invited to participate in the study, of which 422 (92.3%) consented to participate and completed all required study procedures and provided urine, stool and CVL samples for the detection of *Schistosoma* DNA.

South Africa. *Schistosoma* PCR data from a study conducted during 2010–2012 among young women aged 16 to 23 years living in rural KwaZulu-Natal (region 2) were included. In an earlier study in this region the *S. haematobium* prevalence was found to be 31.8% among girls aged 10–12 years, based on urine microscopy analyzed over three consecutive days (Hegertun et al., 2013; Pillay et al., 2014).

Madagascar. Finally, we used *Schistosoma* PCR data from a study conducted in 2010 among rural farming communities across five villages in the district of Miandrivazo in the western part of Madagascar (Randrianasolo et al., 2015). One of these villages was located in a region with a prevalence of *S. haematobium* below 20% (region 3A), while the four other villages were situated within a region hyper-endemic for *S. haematobium*, with a prevalence above 50% (region 3B) (Randrianasolo et al., 2015). In total 38-urine microscopy negative women from the low *S. haematobium* transmission region 3A were included. Within the hyper-endemic villages, 79 women, ranging in age from 15–33 years (median 20 years) were selected based on the average urinary *S. haematobium* egg count in three consecutive urine sample: high-intensity, i.e. $>$ 50 eggs per 10 mL of urine (n = 40), or low-intensity, i.e. $<$20 eggs per 10 mL of urine (n = 39). Part of the microscopy and PCR data has been published before (Randrianasolo et al., 2015).

Participants from the Tanzanian and Malagasy regions were requested to provide a single urine and stool sample on the day of the gynecological examination. Urine and stool samples, were examined for *Schistosoma* eggs by experienced microscopists, using, a urine filtration procedure and a Kato-Katz smear examination respectively, as described previously (Downs et al., 2011; Randrianasolo et al., 2015). In the South African study, participants were requested to provide a single urine sample, of which two aliquots of 10 mL each was examined for *Schistosoma* eggs by experienced microscopists as previously described (Pillay et al., 2016). No stool samples were examined, as *S. mansoni* was found to be absent in the region after performing microscopy and real-time PCR analysis on approximately 600 stool samples from young school girls (Hegertun et al., 2013; Pillay et al., 2014).

In the South African and Malagasy studies, Pap smears were examined by cytologists for the presence of *Schistosoma* eggs in addition to other cytological entities (Pillay et al., 2016; Randrianasolo et al., 2015). In Tanzania, cervical smears were stained with 0.5% Trypan Blue and examined for the presence of *Schistosoma* eggs. Cervical cancer screening was performed using acetic acid.

In all five-study regions, CVL samples were collected during gynecological examination. Sampling was done by washing the surface of the cervix with 10 mL normal saline and collecting 2 mL aliquots. Additionally an aliquot of 1 to 2 mL of urine and 1–2 g of each stool sample was transferred to a cryotube on the day of collection and together with the CVL aliquots stored at either -20 °C or -80 °C. These samples were transported frozen to the Netherlands for further *Schistosoma* DNA testing (Downs et al., 2013; Pillay et al., 2016; Randrianasolo et al., 2015).

2.2. DNA isolation and detection

DNA isolation and PCR reaction set-up steps were performed using a custom-made high throughput Hamilton robot platform (Hamilton Robotics GmbH, Germany) at the Leiden University Medical centre in the Netherlands. Full details on the DNA isolation procedures and set-

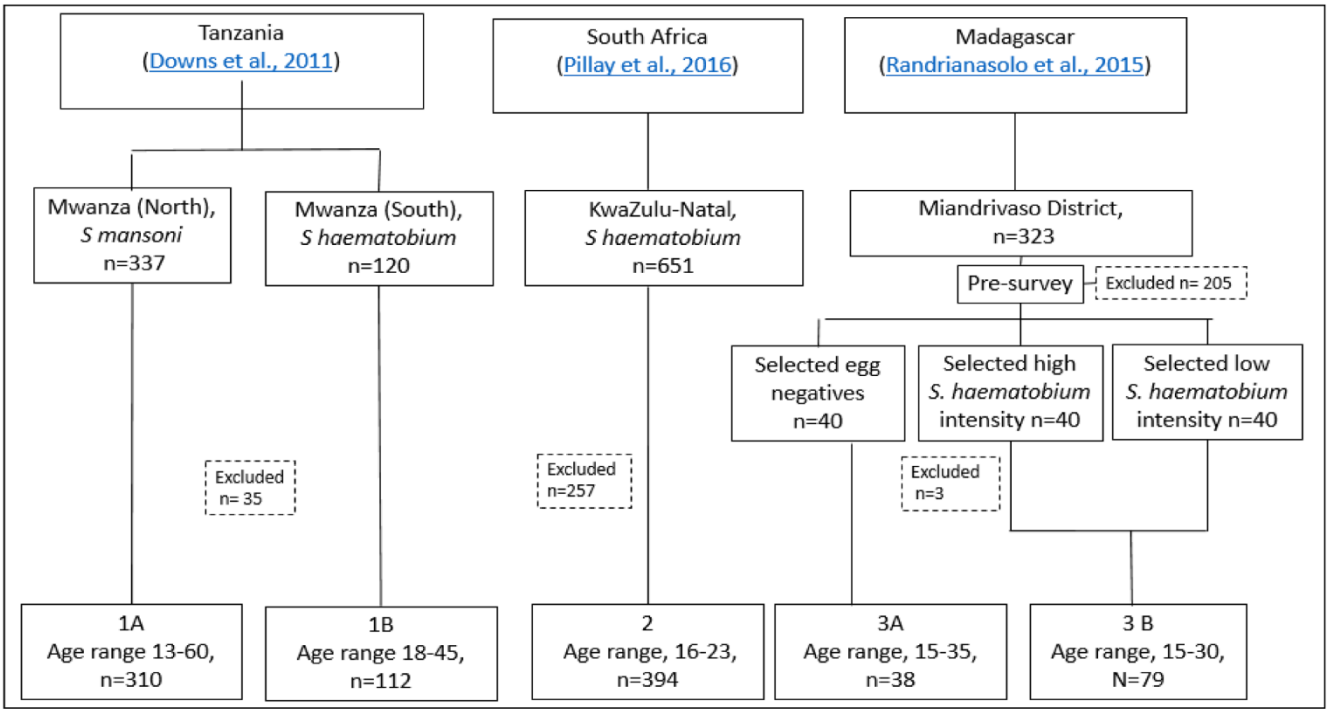

Fig. 1. Participation flow and sampling among the 5 *Schistosoma* endemic regions. All 5 study areas performed urine, vaginal lavage and cervical smear analysis. Stool analysis were only done in Tanzania and Madagascar, not in the South Africa study. (Downs et al., 2011; Pillay et al., 2016; Randrianasolo et al., 2015).

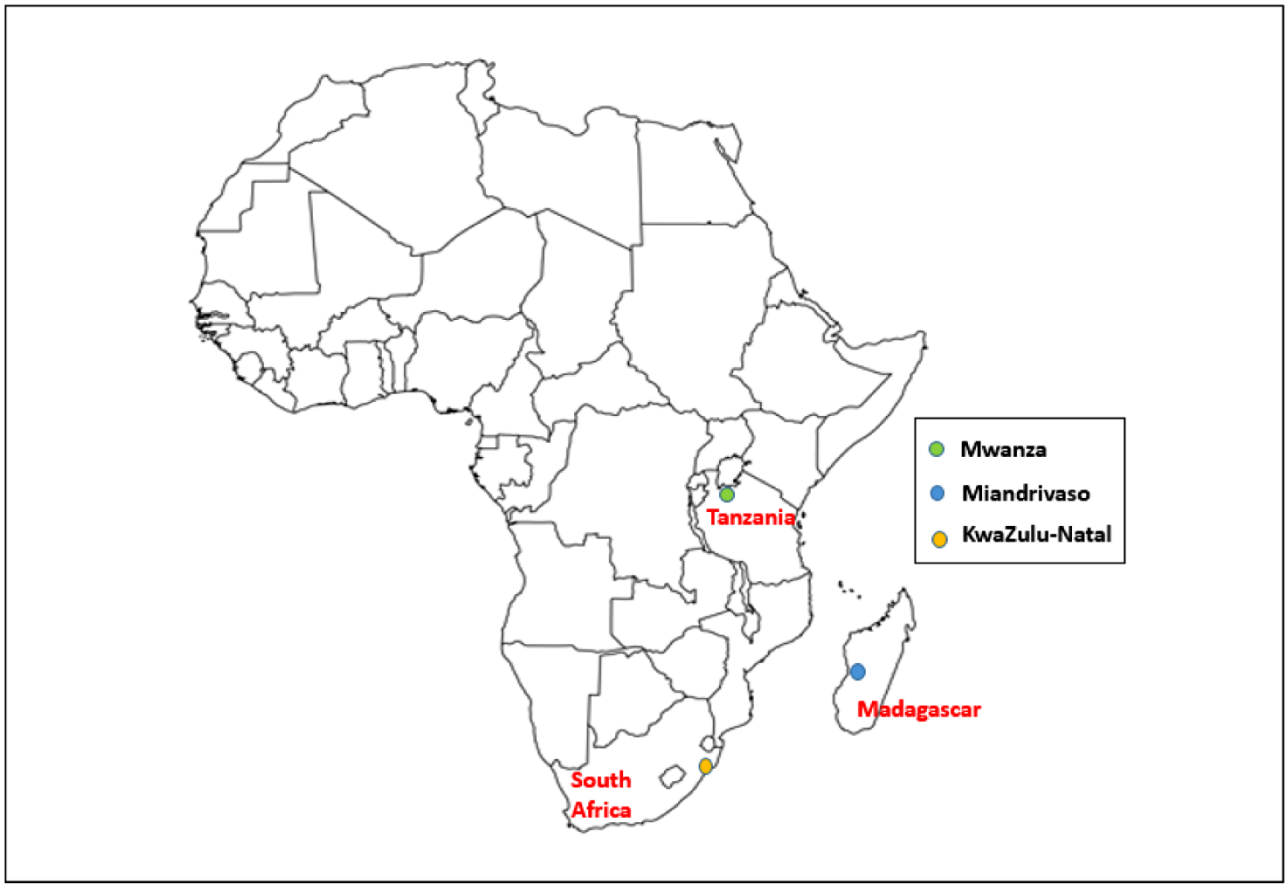

Fig. 2. Map showing the three countries in the south-eastern region of Africa, Tanzania, Madagascar and South Africa with the respective study areas in Mwanza, Miandivaso and KwaZulu-Natal. (Downs et al., 2011; Pillay et al., 2016; Randrianasolo et al., 2015).

up of the multiplex real-time PCR for semi-quantitative detection of the *Schistosoma* genus-specific internal-transcribed-spacer-2 (ITS2) target were as previously described (Downs et al., 2013; Meurs et al., 2015; Obeng et al., 2008). In brief, DNA was isolated using DNeasy 96 Blood & Tissue Kit spin columns and Phocin Herpes Virus-1 (PhHV-1) was added to the lysis buffer in each sample as an internal control. Negative and positive control samples for each parasite species were included in each PCR run. A CFX96 real-time detection system (Bio-Rad laboratories) was used for DNA amplification and detection. Cycle threshold (Ct) value results were analyzed using Bio-Rad CFX software (Manager V1.6.541.1028). These Ct-values represent the amplification cycle in which the level of fluorescent signal exceeds the background fluorescence, reflecting the parasite-specific DNA load in the tested sample. The amplification was considered to be hampered by inhibitory factors if the expected Ct value of 33 in the PhHV-specific PCR was increased by more than 3.3 cycles. *Schistosoma* DNA intensity was arbitrarily classified as either high-intensity ($Ct < 30$), medium-intensity ($30 \leq Ct < 35$), low intensity ($35 \leq Ct < 50$) or negative ($Ct = 50$) (Pillay et al., 2014). This test does not differentiate between *Schistosoma* species.

2.3. Data analysis and statistical testing

PCR analysis was performed blinded from microscopy or other data. The results of the real-time PCR analysis were stored and grouped in a Microsoft Access database and imported into IBM SPSS 20.0 (Chicago, Illinois, USA) for statistical analysis. Descriptive statistics were used in this explorative study. Categorical variables were summarized by frequency and percentage and presented using bar charts. Proportions were compared using Fisher's exact test and McNemar statistical test. Continuous variables for the diagnostic tests were summarized by the median of all positive values and their interquartile range (IQR), or total range when less than 10. Continuous variables were compared between groups by the Mann-Whitney rank-sum test, using the positive values only. Two-sided hypotheses were assumed for all confidence intervals and *P*-values. Statistical significance was set at *P* values less than 0.05.

3. Results

In Table 1, the diagnostic performance regarding the prevalence of urogenital and intestinal schistosomiasis using different tests performed on urine, stool, CVL and Pap-smears is summarized. Fig. 3 depicts the percentage of positives per test per five-study area, according to arbitrary categories of intensity.

3.1. Tanzania urine results

In the *S. mansoni* area in Tanzania (1A), *S. haematobium* eggs in urine were found in 4 (1%) of the 310 women, all with low intensity in the range of 2–12 eggs/10 mL. In the *S. haematobium* endemic area (1B) 13 (12%) of the 112 women were positive in urine microscopy, with intensities of 1–54 eggs/10 ml. Both regions showed more positive results by urine PCR than by microscopy. Table 1 shows that there was a higher prevalence of detectable *Schistosoma* DNA using urine PCR than by microscopy. Also *Schistosoma* DNA was more often detectable in urine from the predominantly *S. haematobium* southern region (20%) than from the predominantly *S. mansoni* endemic northern region (8%). Although the urines from the *S. haematobium* region showed higher DNA loads, reflected by a lower median Ct-value, the difference was not significant in comparison with the urines from the *S. mansoni* region (Table 1).

3.2. Tanzania stool results

S. mansoni eggs were seen at region 1A in 12% ($n = 38$) of the 310 Kato-smears, with a majority showing less than 100 epg, while none of the women from region 1B were found to have *Schistosoma* eggs in stool (Table 1). However, by PCR in stool DNA was found in 10/112 cases of the *S. haematobium* endemic area (9%) with low *Schistosoma* DNA loads (Table 1, Fig. 3). Nine of these 10 women with detectable *Schistosoma* DNA in stool had no detectable *Schistosoma* DNA in urine. Overall *Schistosoma* DNA loads in stool were significantly higher in the *S. mansoni* endemic northern region (1A) than in the *S. haematobium* endemic southern region ($P < 0.05$).

Table 1
Laboratory assays for *Schistosoma* species in genital, urinary and stool samples of 933 women in five schistosomiasis endemic regions. (Downs et al., 2011; Pillay et al., 2016; Randrianasolo et al., 2015)

n = 933			Tanzania Mwanza (North) Region 1A *S mamoni* n = 310	Mwaznu (South) Region 1B *S haematobium* n = 112	South Africa KwaZulu-Natal Region 2 *S haematobium* n = 394	Madagascar Miandriva&o District Region 3A *S haematobium* n = 38	Region 3B *S haemotobium* n = 79
Microscopy	Urine (e/IOmL)	*S. haematobium* positive	4(1.3%)	13 (11.6%)	78 (19.8%)	0	79(100%)
		Range	2-12	1-54	1-71	0%	1-2463
		Median	5	8	13	0	51
		interquartile Range	Not done	5-20	4-37	Not done	4-266
	Stool EPG	*S. monsoni* positive	38 (12.3%)	0	Not done	1 (2.6%)	0
		Range	12-917	0	Not done	1.0-1.0	0%
		Median	36	0	Not done	1	0
		Interquartile Range	12-85	0	Not done	Not done	Not done
PCR	Urine	*Schistosoma* positive	25 (8.1%)	22(19.6%}	91 (23.1%]	5(13.2%)	64 (81.0%)
		Range	20.6-40.4	21.4-36.3	19.2-40.2	37.0-38.0	18.3-45.0
		Median	35.1	32.6	28.6	37.4	25.0
		Interquartile Range	31.2-35.9	26.3-35.1	24.9-34.1	Not done	21.4-23.8
	Stool	*Schistosoma* positive	104 (33.5%)	10 (8.9%)	Not done	I8 (47.4%)	35(44.3%)
		Range	19.1-47.3	23.4-36.5	Not done	20.6-44.6	19.8-44.3
		Median	28.5	34.2	Not done	36.2	32.1
		Interquartile Range	23.9-34.2	29.0-35.0	Not done	35.6-37.4	26.8-36.7
	CVL	*Schistosoma* positive	9 (2.9%)	18(16.1%)	38(9.6%)	7 (18.4%}	48 (60.8%)
		Range	29.5-47.1	21.3-35.8	19.5-37.4	23.1-44.3	18.6-38.3
		Median	34.9	33.9	31.0	35.6	24.7
		Interquartile Range	Not done	30.4-35.0	25.4-34.8	26.8-37.0	223-34.8
Cytology	Pap	*Schistosoma* positive	5 (1.6%]	1 (0.9%)	8 (2.0%}	4 (10.5%)	41 (51.9%)

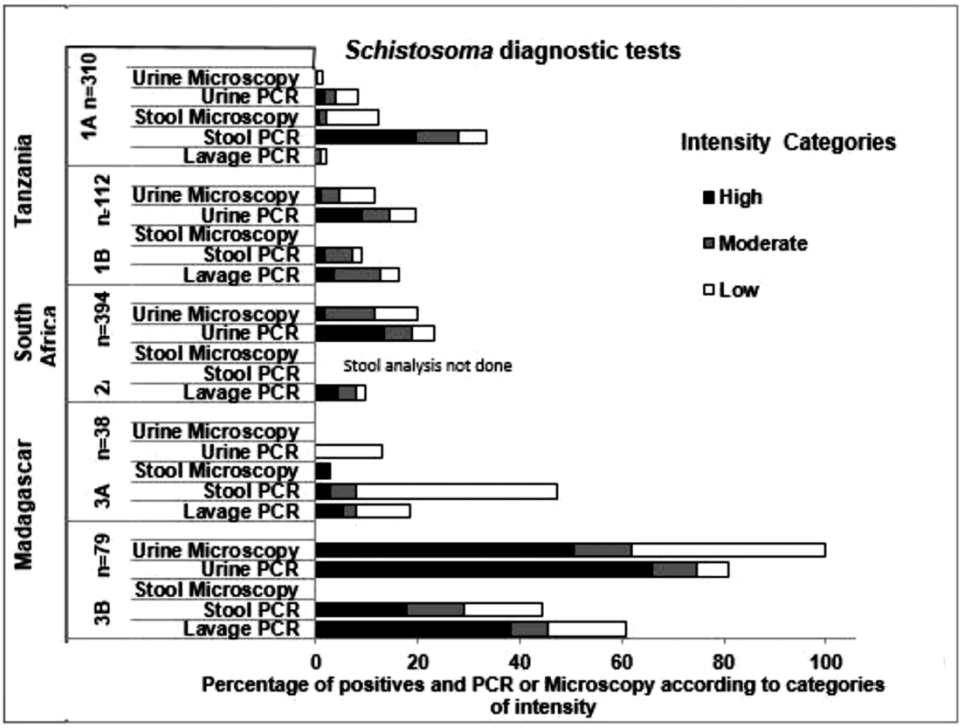

Fig. 3. Summary of *Schistosoma* results according to egg intensities and DNA loads among the 5 study populations. For ease of presentation, the results have been arbitrarily categorised as high, moderate and low intensity of infection. Intensity in urine samples was defined as follows; high intensity >50 ova per 10 mL, moderate intensity as 10–50 ova per 10 mL and low intensity as <10 ova per 10 mL. High intensity of ova in stool samples was defined as >400 ova per gram (epg), moderate intensity as 100–400epg and low intensity as <100epg. The *Schistosoma* DNA loads were defined as high-intensity (Ct < 30), medium-intensity (30 ≤ Ct < 35) or low intensity (35 ≤ Ct < 50) 1A = *S. mansoni* endemic area, 1B = *S. haematobium* endemic area, 2 = *S. haematobium* endemic area, 3A = low-endemic *S. haematobium* area, all *S. haematobium* negative, 3B = = high-endemic *S. haematobium* area, all *S. haematobium* positive.

3.3. Tanzania lavage results

CVL PCR analysis showed detectable *Schistosoma* DNA in 9/310 (3%) of the women in the *S mansoni* endemic region (1A) compared to 18/112 (16%) of the women in the *S. haematobium* endemic region (1B). Six of these nine women of region 1A also had detectable *Schistosoma* DNA in their stool samples, while their urines were PCR and microscopy negative. Despite the difference in prevalence of positive CVL by PCR, *Schistosoma* DNA loads were comparable between the two regions (Table 1). However the sample size was very low and this might represent a type 2 error.

3.4. Comparison between CVL and urine results for all study regions

Fig. 4 depicts the relation between the CVL PCR and urine microscopy or urine PCR for the examined Tanzania population, in comparison to the study populations of South Africa and Madagascar. For each tested population, the prevalence based on the detection of *Schistosoma* DNA in urine was higher than the prevalence based on urine microscopy, with the exception of Madagascar 3B where 100% were microscopy positive (as per inclusion criteria, Table 1). Notably, 15 women of the Madagascar 3B group had a negative PCR in urine (Table 1). They all belonged to the group preselected based on a positive urine microscopy of less than 20 eggs/10 mL. The median egg excretion of these 15 was 3eggs/10 mL. Overall, the PCR in urine seems to be a better proxy than urine microscopy for the presence of *Schistosoma* DNA in lavage. Notably, the 15 women of Madagascar with a negative urine PCR all belonged to a preselected low-endemic village and although the Malagasy women had submitted three urines the actual urine tested for by PCR was fourth urine on the investigation day. In South Africa only one urine sample per participant was tested, yet the PCR and urine microscopy results are similar.

3.5. Relation to cytology outcome

Schistosoma eggs were demonstrated on cytological examination of 5/310 (2%) Tanzanian women of the *S. mansoni* region 1A, compared to 1/112 (1%) woman of the *S. haematobium* endemic region 1B (Table 1). None of these six women were positive in the CVL PCR. In contrast, in the 394 women in South Africa, *Schistosoma* DNA was detected in the lavages of all 8 egg-positive Pap-smear cases. The DNA levels of these 8 were significantly higher than the lavage DNA levels of the 30 CVL PCR positive women negative in the cytology ($P < 0.001$).

In the low endemic region of Madagascar (region 3A) Pap-smear examination revealed 4 cases with detectable eggs among the 38 urine microscopy negative women. One of the 4 women also showed *Schistosoma* DNA in CVL. In the 79 *S. haematobium* infected women from region 3B, *Schistosoma* DNA was demonstrated in CVL in 28 (68%) of the 41 women with egg-positive Pap smears. Similar to the South African region, the lavage DNA levels of these 28 women were significantly higher than the lavage DNA levels of the 20 women negative in the cytology ($P < 0.001$).

4. Discussion

The quest for FGS diagnostic tools with high performance level continues as more information on this neglected entity unfolds. A single diagnostic test for FGS would be ideal, however such a single applicable test is currently not yet available and a panel of laboratory and complementary tests are still required due to the complexity of this disease (Christinet et al., 2016). The diagnosis of FGS is complex since it occurs as a result of morbidity caused by ova in genital tissue, meaning that finding ova in urine neither confirms nor refutes the presence of genital involvement (Galappaththi-Arachchige et al., 2018; Jourdan et al., 2013). In this study, the outcome of real-time PCR on CVL was compared with microscopy and PCR analysis of stool and urine samples from 933 women, living in three different Sub-Saharan countries. The only available FGS reference test for these samples was cytology on Pap-smear, a procedure which is not highly standardized and is known for its low sensitivity for egg detection (Kjetland et al., 2005; Poggensee et al., 1999).

While traditional microscopy is cheaper and more readily available, it also poses challenges like observer variation, day-to-day variation in egg excretion, and reduced sensitivity in detecting low intensity infections. Similar to previous studies we found more schistosomiasis cases by detection of *Schistosoma* DNA in urine via PCR than by microscopy

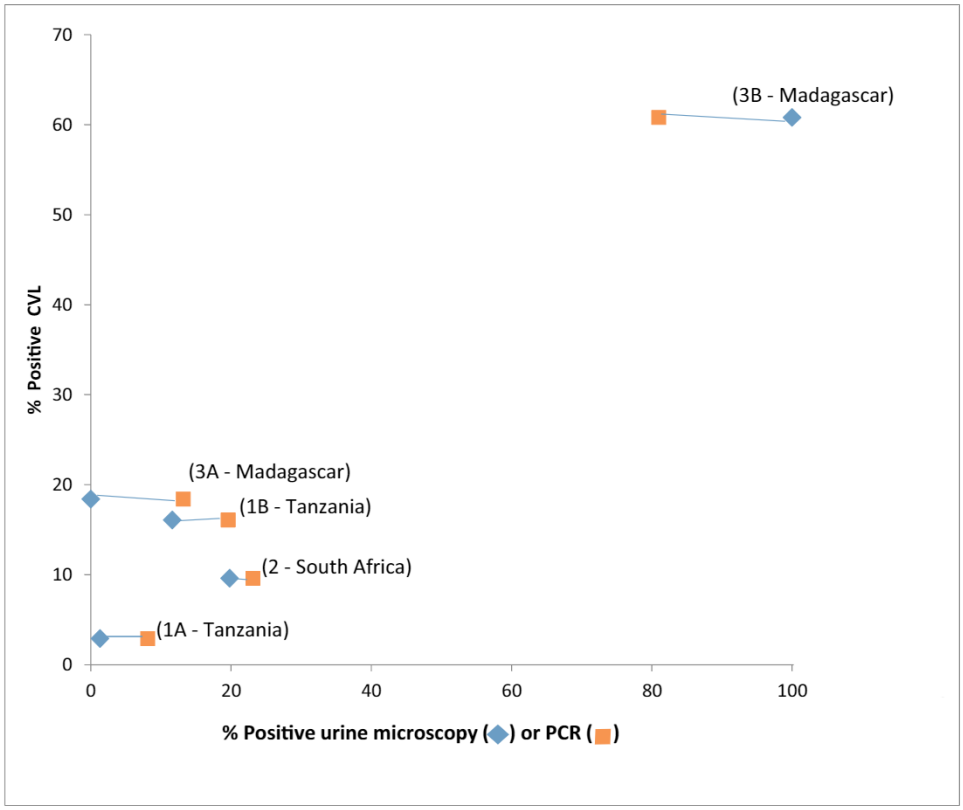

Fig. 4. Combined analysis of data in three countries from five sites with different prevalence's. Relation between prevalence of urinary schistosomiasis and the occurrence of genital schistosomiasis (PCR of cervicovaginal lavage) in women with urinary schistosomiasis (urine PCR (red square) and urine microscopy (blue diamond)). 1A= *S. mansoni* endemic area, 1B= *S. haematobium* endemic area, 2 = *S. haematobium* endemic area, 3A= low-endemic *S. haematobium* area, all *S. haematobium* negative, 3B= high-endemic *S. haematobium* area, all *S. haematobium* positive (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

(Cavalcanti et al., 2013; Obeng et al., 2008). In addition, and in conformance with previous studies, those who were PCR negative but microscopy positive showed very low egg counts, such as the 15 women of Madagascan high-endemic site (3B) who only had a median intensity of 3 eggs/10 mL of urine based on three consecutive microscopy examinations. While PCR has better specificity and sensitivity, and could potentially be applied on other types of clinical samples such as vaginal swabs or sanitary pads, it has the disadvantage of high cost, and low field applicability (Cavalcanti et al., 2013). However, new developments in the direction of field-applicable DNA detection seem promising (Poulton and Webster, 2018; Rosser et al., 2015). An additional limitation with urine PCR is that in chronic infections, *Schistosoma* granulomas formed in the urinary bladder do not always enable egg excretion and such infections may remain undiagnosed (Shiff et al., 2006).

Women may have urinary schistosomiasis with or without genital involvement (Galappaththi-Arachchige et al., 2018; Poggensee et al., 1998; Swai et al., 2006). For example, in the South African study, an overall correlation was seen between the outcome of the PCR on urine and on CVL, but at the same time some individual cases had detectable *Schistosoma* DNA in CVL only, supporting the notion that schistosomiasis could occur in the genital tract without involving the urinary tract (Galappaththi-Arachchige et al., 2018; Pillay et al., 2016). In this study, we also found PCR on urine to be a better indicator of the presence of *Schistosoma* DNA in CVL, than urine microscopy. However, it should be mentioned that the overall intensity of *S. haematobium* infection in our study population was low. Only 47 of the 933 included women showed more than 50 eggs/10 mL of urine, 40 of them from the Madagascar 3B region. Supplementary studies from different *S. haematobium* regions with distinctive transmission patterns might shed new light on the relation between urinary schistosomiasis and the presence of *Schistosoma* DNA in CVL. Studies have shown that genetic variations exist among the *S. haematobium* species and this may have bearing on many factors including the clinical outcomes, which could possibly explain the differences in parasite detection rates between urine and CVL samples (Ezeh et al., 2015; Quan et al., 2015).

Schistosoma DNA in CVL was detected in all 5-study regions. However, the agreement in the prevalence of positive cases and observed DNA levels varied substantially between the different groups. Detection of *Schistosoma* DNA using CVL PCR was generally higher than rates of egg detection by Pap-smears. Despite Pap-smears being a relatively cheap diagnostic tool, they have limitations especially in developing countries (Pillay et al., 2016). The procedure is operator and observer dependent, requires trained laboratory personnel and is not standardized. The finding that CVL DNA levels were significantly higher in the Pap -smear positive women of South Africa and Madagascar, but not in Tanzania seems to suggest poorer quality of the smear cytology in the Tanzanian setting where smears were stained with Trypan blue and read in the field. On the other hand, in Madagascar, not all Pap-smear positives could be PCR confirmed.

Whether PCR outcomes, in particular the quantitative output, still can be compared when performed in different laboratories using slightly different PCR procedures needs to be further explored. The start of an external quality assessment scheme, using a set of well-defined clinical samples as a proficiency panel, would be a first step into further harmonization of PCR-based diagnosis of schistosomiasis. Such a scheme already exists for other parasites for example protozoa and has recently been launched for helminths (Schuurs et al., 2018). At least, our PCR was performed under the same conditions in the same laboratory, while microscopy procedures clearly differed. In South Africa, urine was collected once and our PCR showed that the assumed *S. haematobium* negative group in Madagascar was not truly negative.

We have not included clinical investigations as an indicator of FGS as there were at least 7 different clinicians in the sites and the work started before the WHO Pocket Atlas for FGS was released (Norseth et al., 2014). Furthermore, there was a notable difference in age between the different regions studied, with mainly young women only in the South African study population and a broader age range in the other populations. Although no effect of age on the PCR outcome was noticed, this could be further explored in future studies. For this purpose a

prospective study should be designed, including highly standardized clinical parameters of FGS to investigate the association between FGS lesions, age of the female population and *Schistosoma* DNA in CVL and urine in different geographical regions.

The *S. haematobium* worm is known to prefer residence in the pelvic venous plexuses therefore the ova tend to become lodged in urogenital tissues, while *S. mansoni* prefers the portal venous system and is usually found in the intestinal tract (Colley et al., 2014). The detection of *Schistosoma* DNA in CVL and stool samples, while being negative in the *Schistosoma* PCR in urine, might suggest that not only S. haematobium, but also *S mansoni* could lead to FGS. Alternatively, this could signify that *S. haematobium* can be present in the intestinal tract without being present in the urine, as is the case for the genital tract (Al-Adnani and Saleh, 1982; Tzanetou et al., 2010). While there is evidence that *S. haematobium* is the main causative agent for FGS, little is known about the effect of *S. mansoni* (Poggensee et al., 1998, 2001a).

In recent case reports from Brazil *S. mansoni* has been found in the fallopian tubes and ovaries (Arruda et al., 2007; Cavalcanti et al., 2013; Gonçalves Amorim et al., 2014). *S. mansoni* eggs have also been described in the cervix in a case series from another region of Tanzania, and autopsy studies in Egypt reported that *S. mansoni* eggs were distributed throughout the urogenital system, though at a lower density than *S. haematobium* eggs (Gelfand and Ross, 1953; Kamel et al., 1977; Poggensee et al., 2001a). In regions in Africa, it has been found that there is overlap between the geographical areas of *S. mansoni* and *S. haematobium* species and patients are at risk for dual infection (Meurs et al., 2012). The finding of stool positive PCR samples in the *S. haematobium* area might suggest mono-infections with the *S. mansoni* species although one cannot preclude intestinal *S. haematobium*, a fairly common condition. Still, most epidemiological and diagnostic studies seem to focus on one of the two species only. With the advent of migration and travelling, it is recommended that at least at population level, *Schistosoma* species-specific PCR screening tests are used to accommodate possible overlaps between the species and to provide more species-specific information on the clinical outcomes (Cnops et al., 2012; Sady et al., 2015).

5. Conclusion

This explorative descriptive study further supports the diagnostic potentials of real-time PCR for the detection of *Schistosoma* DNA in clinical samples. While PCR performed on stool or urine samples seems most useful as a form of quality control to counter-check microscopy results, or perhaps as a tool for mapping and or monitoring the disease at a population level, the PCR performed on CVL is a potential as a diagnostic test for individual FGS cases. Detection of *Schistosoma* DNA has the potential of being a more sensitive diagnostic technique for FGS in the absence of a non-invasive true gold standard for FGS diagnosis, in particular among women living in low resource settings who are also at risk for HIV and cervical cancer.

Funders

The research leading to these results has received funding from the European Research Council under the Seventh Framework Programme, ERC Grant agreement no. PIRSES-GA-2010-269245, University of Copenhagen with the support from the Bill and Melinda Gates Foundation grant no. OPPGH5344, Norwegian Research Council ref. 213702/H10, the South Eastern Regional Health Authority, Norway (no. 2016055, 2011073, 2012032), National Research Foundation SGD14052367807.

The Tanzania project was supported by the 2009 Merle A. Sande/Pfizer Fellowship Award in International Infectious Diseases, awarded annually by the Infectious Diseases Society of America Education and Research Foundation and the National Foundation for Infectious Diseases. It was also supported by NIH AHRQ T32 HS000066 and the Weill Cornell Clinical and Translational Science Center Grant (UL1-RR024996, REDCap).

CRediT authorship contribution statement

P. Pillay: Writing - original draft, Validation, Writing - review & editing. **J.A. Downs:** Investigation, Resources, Writing - review & editing, Supervision. **J.M. Changalucha:** Validation, Resources. **E.A.T. Brienen:** Validation, Resources. **C.E. Ramarokoto:** Validation, Resources. **P.D.C. Leutscher:** Investigation, Resources, Writing - review & editing. **B.J. Vennervald:** Writing - review & editing. **M. Taylor:** Supervision, Writing - review & editing. **E.F. Kjetland:** Supervision, Writing - review & editing. **L. Van Lieshout:** Supervision, Conceptualization, Data curation, Methodology, Writing - original draft, Writing - review & editing.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

All co-authors of Madagascan, Tanzanian and South African papers and study participants.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2020.105363.

References

Al-Adnani, M., Saleh, K., 1982. Extraurinary schistosomiasis in Southern Iraq. Histopathology 6, 747–752.
Arruda, R.M., Castro, R., Tamura, M.V., Ruano, J.M., Baracat, E.C., Girão, M., 2007. Mansoni's schistosomiasis of a leiomyoma: case report. Clinics 62, 529–530.
Cavalcanti, M.G., Silva, L.F., Peralta, R.H., Barreto, M.G., Peralta, J.M., 2013. Schistosomiasis in areas of low endemicity: a new era in diagnosis. Trends Parasitol. 29, 75–82.
Christinet, V., Lazdins-Helds, J.K., Stothard, J.R., Reinhard-Rupp, J., 2016. Female genital schistosomiasis (FGS): from case reports to a call for concerted action against this neglected gynaecological disease. Int. J. Parasitol. 46, 395–404.
Clements, A.C., Lwambo, N.J., Blair, L., Nyandindi, U., Kaatano, G., Kinung'hi, S., Webster, J.P., Fenwick, A., Brooker, S., 2006. Bayesian spatial analysis and disease mapping: tools to enhance planning and implementation of a schistosomiasis control programme in Tanzania. Trop. Med. Int. Health 11, 490–503.
Cnops, L., Tannich, E., Polman, K., Clerinx, J., Van Esbroeck, M., 2012. *Schistosoma* real-time PCR as diagnostic tool for international travellers and migrants. Trop. Med. Int. Health 17, 1208–1216.
Colley, D.G., Bustinduy, A.L., Secor, W.E., King, C.H., 2014. Human schistosomiasis. The Lancet 383, 2253–2264.
Downs, J.A., Dupnik, K.M., van Dam, G.J., Urassa, M., Lutonja, P., Kornelis, D., Claudia, J., Hoekstra, P., Kanjala, C., Isingo, R., Peck, R.N., Lee, M., Corstjens, P.L.A.M., Todd, J., Changalucha, J.M., Johnson, W.D., Fitzgerald, D.W., 2017. Effects of schistosomiasis on susceptibility to HIV-1 infection and HIV-1 viral load at HIV-1 seroconversion: a nested case-control study. PLoS Negl. Trop. Dis. 11, e0005968.
Downs, J.A., Kabangila, R., Verweij, J.J., Jaka, H., Peck, R.N., Kalluvya, S.E., Changalucha, J.M., Johnson, W.D., van Lieshout, L., Fitzgerald, D.W., 2013. Detectable urogenital schistosome DNA and cervical abnormalities 6 months after single-dose praziquantel in women with *schistosoma haematobium* infection. Trop. Med. Int. Health 18, 1090–1096.
Downs, J.A., Mguta, C., Kaatano, G.M., Mitchell, K.B., Bang, H., Simplice, H., Kalluvya, S.E., Changalucha, J.M., Johnson Jr, W.D., Fitzgerald, D.W., 2011. Urogenital schistosomiasis in women of reproductive age in Tanzania's Lake Victoria region. Am. J. Trop. Med. Hyg. 84, 364–369.
Ezeh, C., Yin, M., Li, H., Zhang, T., Xu, B., Sacko, M., Feng, Z., Hu, W., 2015. High genetic variability of Schistosoma haematobium in Mali and Nigeria. Korean J. Parasitol. 53, 129.
Galappaththi-Arachchige, H.N., Holmen, S., Koukounari, A., Kleppa, E., Pillay, P., Sebitloane, M., Ndhlovu, P., Van Lieshout, L., Vennervald, B.J., Gundersen, S.G., Taylor, M., Kjetland, E.F., 2018. Evaluating diagnostic indicators of urogenital *Schistosoma haematobium* infection in young women: a cross sectional study in rural South Africa. PLoS ONE 13, e0191459.

Gelfand, M., Ross, W., 1953. II. the distribution of schistosome ova in the genito-urinary tract in subjects of bilharziasis. Trans. R. Soc. Trop. Med. Hyg. 47, 218–220.
Gonçalves Amorim, A., Alves Barbosa Pagio, F., Neves Ferreira, R., Chambô Filho, A., 2014. Genital schistosomiasis: a report on two cases of ovarian carcinomas containing viable eggs of *Schistosoma mansoni*. Case Reports in Obstetrics and Gynecology 2014.
Hegertun, I.E.A., Gundersen, K.M.S., Kleppa, E., Zulu, S.G., Gundersen, S.G., Taylor, M., Kvalsvig, J.D., Kjetland, E.F., 2013. *S. haematobium* as a common cause of genital morbidity in girls: a cross-sectional study of children in South Africa. PLoS Negl. Trop. Dis. 7, e2104.
Holmen, S.D., Kleppa, E., Lillebø, K., Pillay, P., Van Lieshout, L., Taylor, M., Albregtsen, F., Vennervald, B.J., Onsrud, M., Kjetland, E.F., 2015. The first step toward diagnosing female genital schistosomiasis by computer image analysis. Am. J. Trop. Med. Hyg. 93, 80–86.
Jourdan, P.M., Randrianasolo, B.S., Feldmeier, H., Chitsulo, L., Ravoniarimbinina, P., Roald, B., Kjetland, E.F., 2013. Pathologic mucosal blood vessels in active female genital schistosomiasis: new aspects of a neglected tropical disease. Int. J. Gynecol. Pathol. 32, 137–140.
Kamel, I.A., Cheever, A.W., Elwi, A.M., Mosimann, J.E., Danner, R., 1977. *Schistosoma Mansoni* and *S. haematobium* infections in Egypt. Am. J. Trop. Med. Hyg. 26, 696–701.
Kjetland, E.F., Leutscher, P.D., Ndhlovu, P.D., 2012. A review of female genital schistosomiasis. Trends Parasitol. 28, 58–65.
Kjetland, E.F., Ndhlovu, P.D., Gomo, E., Mduluza, T., Midzi, N., Gwanzura, L., Mason, P.R., Sandvik, L., Friis, H., Gundersen, S.G., 2006. Association between genital schistosomiasis and HIV in rural Zimbabwean women. Aids 20, 593–600.
Kjetland, E.F., Ndhlovu, P.D., Mduluza, T., Gomo, E., Gwanzura, L., Mason, P.R., Kurewa, E.N., Midzi, N., Friis, H., Gundersen, S.G., 2005. Simple clinical manifestations of genital *Schistosoma haematobium* infection in rural Zimbabwean women. Am. J. Trop. Med. Hyg. 72, 311–319.
Meurs, L., Brienen, E., Mbow, M., Ochola, E.A., Mboup, S., Karanja, D.M., Secor, W.E., Polman, K., van Lieshout, L., 2015. Is PCR the next reference standard for the diagnosis of schistosoma in stool? A comparison with microscopy in Senegal and Kenya. PLoS Negl. Trop. Dis. 9, e0003959.
Meurs, L., Mbow, M., Vereecken, K., Menten, J., Mboup, S., Polman, K., 2012. Epidemiology of mixed *Schistosoma mansoni* and *Schistosoma haematobium* infections in northern Senegal. Int. J. Parasitol. 42, 305–311.
Norseth, H.M., Ndhlovu, P.D., Kleppa, E., Randrianasolo, B.S., Jourdan, P.M., Roald, B., Holmen, S.D., Gundersen, S.G., Bagratee, J., Onsrud, M., Kjetland, E.F., 2014. The colposcopic atlas of schistosomiasis in the lower female genital tract based on studies in Malawi, Zimbabwe, Madagascar and South Africa. PLoS Negl. Trop. Dis. 8, e3229.
Obeng, B., Aryeetey, Y., De Dood, C., Amoah, A., Larbi, I., Deelder, A., Yazdanbakhsh, M., Hartgers, F., Boakye, D., Verweij, J., Van Dam, G.J., Van Lieshout, L., 2008. Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of *Schistosoma haematobium* in urine samples from Ghana. Ann. Trop. Med. Parasitol. 102, 625–633.
Pillay, P., Taylor, M., Zulu, S.G., Gundersen, S.G., Verweij, J.J., Hoekstra, P., Brienen, E.A., Kleppa, E., Kjetland, E.F., Van Lieshout, L., 2014. Real-time polymerase chain reaction for detection of *Schistosoma* DNA in small-volume urine samples reflects focal distribution of urogenital Schistosomiasis in primary school girls in Kwazulu Natal, South Africa. Am. J. Trop. Med. Hyg. 90, 546–552.
Pillay, P., Van Lieshout, L., Taylor, M., Sebitloane, M., Zulu, S.G., Kleppa, E., Roald, B., Kjetland, E.F., 2016. Cervical cytology as a diagnostic tool for female genital schistosomiasis: correlation to cervical atypia and *Schistosoma* polymerase chain reaction. Cytojournal 13.
Poggensee, G., Feldmeier, H., Krantz, I., 1999. Schistosomiasis of the female genital tract: public health aspects. Parasitol. Today 15, 378–381.
Poggensee, G., Kiwelu, I., Saria, M., Richter, J., Krantz, I., Feldmeier, H., 1998. Schistosomiasis of the lower reproductive tract without egg excretion in urine. Am. J. Trop. Med. Hyg. 59, 782–783.
Poggensee, G., Krantz, I., Kiwelu, I., Diedrich, T., Feldmeier, H., 2001a. Presence of *Schistosoma mansoni* eggs in the cervix uteri of women in Mwanga District, Tanzania. Trans. R. Soc. Trop. Med. Hyg. 95, 299–300.
Poggensee, G., Sahebali, S., Van Marck, E., Swai, B., Krantz, I., Feldmeier, H., 2001b. Diagnosis of genital cervical schistosomiasis: comparison of cytological, histopathological and parasitological examination. Am. J. Trop. Med. Hyg. 65, 233–236.
Poulton, K., Webster, B., 2018. Development of a lateral flow recombinase polymerase assay for the diagnosis of *Schistosoma mansoni* infections. Anal. Biochem. 546, 65–71.
Quan, J.-H., Choi, I.-W., Ismail, H.A.H.A., Mohamed, A.S., Jeong, H.-G., Lee, J.-S., Hong, S.-T., Yong, T.-S., Cha, G.-H., Lee, Y.-H., 2015. Genetic diversity of *Schistosoma haematobium* eggs isolated from human urine in Sudan. Korean J. Parasitol. 53, 271.
Rosser, A., Rollinson, D., Forrest, M., Webster, B., 2015. Isothermal recombinase polymerase amplification (RPA) of *Schistosoma haematobium* DNA and oligochromatographic lateral flow detection. Parasites & vectors 8, 446.
Randrianasolo, B.S., Jourdan, P.M., Ravoniarimbinina, P., Ramarokoto, C.E., Rakotomanana, F., Ravaoalimalala, V.E., Gundersen, S.G., Feldmeier, H., Vennervald, B.J., Van Lieshout, L., Kjetland, E.F., 2015. Gynecological manifestations, histopathological findings, and *schistosoma* -specific polymerase chain reaction results among women with *Schistosoma haematobium* infection: a cross-sectional study in Madagascar. J. Infect. Dis. 212, 275–284.
Sady, H., Al-Mekhlafi, H., Ngui, R., Atroosh, W., Al-Delaimy, A., Nasr, N., Dawaki, S., Abdulsalam, A., Ithoi, I., Lim, Y., Chua, K.H., Surin, J., 2015. Detection of *Schistosoma mansoni* and *Schistosoma haematobium* by real-time PCR with high resolution melting analysis. Int. J. Mol. Sci. 16, 16085–16103.
Schuurs, T.A., Koelewijn, R., Brienen, E.A., Kortbeek, T., Mank, T.G., Mulder, B., Stelma, F.F., van Lieshout, L., van Hellemond, J.J., 2018. Harmonization of PCR-based detection of intestinal pathogens: experiences from the Dutch external quality assessment scheme on molecular diagnosis of protozoa in stool samples. Clin. Chem. Lab. Med. (CCLM) 56, 1722–1727.
Shiff, C., Veltri, R., Naples, J., Quartey, J., Otchere, J., Anyan, W., Marlow, C., Wiredu, E., Adjei, A., Brakohiapa, E., 2006. Ultrasound verification of bladder damage is associated with known biomarkers of bladder cancer in adults chronically infected with *Schistosoma haematobium* in Ghana. Trans. R. Soc. Trop. Med. Hyg. 100, 847–854.
Ssetaala, A., Nakiyingi-Miiro, J., Asiki, G., Kyakuwa, N., Mpendo, J., Van Dam, G.J., Corstjens, P.L., Pala, P., Nielsen, L., Bont, J.D., Pantaleo, G., Kiwanuka, N., Kaleebu, P., Anatoli, K., Elliot, A.M., 2015. *Schistosoma mansoni* and hiv acquisition in fishing communities of Lake Victoria, Uganda: a nested case–control study. Trop. Med. Int. Health 20, 1190–1195.
Swai, B., Poggensee, G., Mtweve, S., Krantz, I., 2006. Female genital schistosomiasis as an evidence of a neglected cause for reproductive ill-health: a retrospective histopathological study from Tanzania. BMC Infect. Dis. 6, 134.
Tzanetou, K., Astriti, M., Delis, V., Moustakas, G., Choreftaki, T., Papaliodi, E., Sarri, K., Adamis, G., 2010. Intestinal schistosomiasis caused by both *Schistosoma intercalatum* and *Schistosoma mansoni*. Travel Med. Infect. Dis. 8, 184–189.
Wall, K.M., Kilembe, W., Vwalika, B., Dinh, C., Livingston, P., Lee, Y.-M., Lakhi, S., Boeras, D., Naw, H.K., Brill, I., Chomba, E., Sharkey, T., Parker, R., Shutes, E., Tichacek, A., Secor, W.E., Allen, S., 2018. Schistosomiasis is associated with incident HIV transmission and death in Zambia. PLoS Negl. Trop. Dis. 12, e0006902.
WHO, 2019. Schistosomiasis, Geneva. Availablehttps://www.who.int/news-room/fact-sheets/detail/schistosomiasis (Accessed Date 2019 Aug 10).